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A RADIOISOTOPE METHOD FOR DETERMINATION OF RATE OF DISAPPEARANCE OF LEAF LITTER IN WOODLAND

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Details are given of an isotope-labelling technique, which enables the recovery of leaves of known age from the forest floor. They are marked whilst still on the tree with a spot of artists' oil colour to which has been added tantalum 182, and are located on the ground with a portable Geiger–Müller detector. Preliminary observations of an experiment to determine the rate of disappearance of beech-leaf litter are given together with a discussion of the method and the effect of gamma radiation on insects and other invertebrate animals

THERE is very little information about the rate of disappearance of leaf litter of tree species from the forest floor, and still less concerning litter of the same species when grown under different conditions. Previous investigations have employed three methods of approach. In the first, litter is placed on the ground in wood or metal containers with a wire-mesh or cloth floor (LUNT, 1935; FALCONER, WRIGHT and BEALL, 1933; OHMASA and MORI, 1937 (on soil in partially buried, earthenware pots); GUSTAFSON, 1943; COLDWELL and DELONG, 1950; HATCH, 1955). The second method consists in placing litter directly on the ground, and covering it with a protective material such as wire netting (JOHNSTON, 1935; WITTICH, 1939; RICHARD, 1945–6), or the leaves are placed in open-mesh wire bags† (JOHNSTON, 1935; NÖMMIK, 1938). MIKOLA (1955) put the litter on a thin layer of glass wool and covered it with the same material. Finally the litter may be sampled at intervals, and recently fallen material sorted from the remainder (see Capstick, p. 353).

All these methods have serious disadvantages. With the first and second, the leaf litter is maintained under conditions which may bear little relationship to those occurring in the natural milieu, and incorporation in the soil may be rendered difficult or impossible. In the case of litter sampling, errors will arise in the sorting process owing to the difficulty of identifying the leaf fall of a particular year. In any case this approach is hardly practicable with species that disappear very slowly. It is felt that at least some of these workers had little idea that animals other than microfauna existed in the forest floor.

Marking leaves with a radioisotope whilst they are still on the tree provides a means for recovery of litter of known age from the ground, and overcomes some of the difficulties already referred to. This technique can

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† Gilbert and Bocock (see p. 348) put the leaves in hair-nets, and this technique overcomes some of the objections referred to in the above paper—Ed.

be used also for determining the distribution of leaf litter, and the effect of such factors as wind on the distribution pattern. The author has also used it to provide litter material of known age for culture experiments in the laboratory.

MARKING METHOD

This technique requires a radioactive material which has a suitable radiation (strong beta or gamma), is chemically relatively inert, and with a half life which permits detection of leaves up to one year after marking. Under field conditions where a portable Geiger–Müller detector is used, a high-energy radiator is essential. It is preferable that the isotope should be easy to handle, and in addition should be easily and readily incorporated with paint. A modification of a method devised by BANKS (1955), where tantalum 182 (half life: 111 days) was used for marking larvae of Coccinellidae, fulfilled these requirements. Tantalum can be obtained as a finely ground powder, and the target element, ^{180}Ta, is quickly irradiated to a suitable activity.

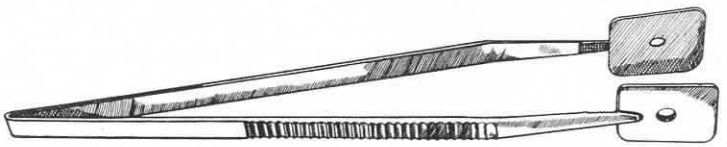

Figure 104. Modified surgical forceps for applying radioactive paint spot.

The tantalum powder was irradiated at the Atomic Energy Research Establishment, Harwell, to provide a specific activity of about 19·5 mc/g. After preliminary trials it was decided to use an artists' oil colour as adhesive. Aureolin (Cobalt yellow)*, a double nitrite of cobalt and potassium, and biologically non-toxic, was chosen for this purpose. A paint spot (6·4 mm diameter; 0·25 in.) with a weight of about 5 mg was used. A weighed quantity of ^{182}Ta was added to provide an average initial activity per spot of about 4·9 μc. Ether was added as solvent to the paint to aid in the mixing with ^{182}Ta. After mixing, the ether was allowed to evaporate, and any further thinning required was obtained by the addition of a small quantity of distilled turpentine.

The method of marking was to grip the leaf on the tree with a large surgical forceps (*l* 25 cm; 10 in.), to the tips of which two chromium-plated blades were fastened (*Figure 104*); in each blade a hole (6·4 mm diameter) was drilled. The glass tube containing the paint was shielded by placing in a lead pot (6 mm (0·25 in.) walls), and a long-handled, oil-painting brush (*l* 30 cm; 12 in.) with short, hog's-hair bristles was used to apply it to the hole in the blade of the forceps. A batch of 50 leaves could be marked in about 10 minutes. A still, dry day was chosen for the marking process to ensure that the paint would dry before nightfall.

DETECTING MARKED LEAVES

The marked leaves are located on the ground with a portable Geiger–Müller detector of a type described by GODFREY (1954). The counter unit employs

* Supplied by Messrs. Winsor and Newton Ltd., Wealdstone, Harrow, Middlesex.

a halogen-quenched G-M tube* (type G10H), with a simple two-stage amplifier connected to a pair of headphones, and operated from a dry battery (*ca.* 400 V); the circuit is illustrated in *Figure 105*. The detector with a weight of about 9·5 lb (4·3 kg) is very portable and has proved a robust and reliable instrument. It has the additional advantage of being very directional.

The method of searching was to enclose a randomly chosen quadrat (1 × 1 m) with an alloy-metal frame, and examine it systematically with the detector. When the approximate position of the marked leaf was found, the litter was carefully removed until it was located. In this way it was possible to determine its exact position in the litter layer. The presence of the coloured spot proved helpful for the final location of the leaf. To assist in

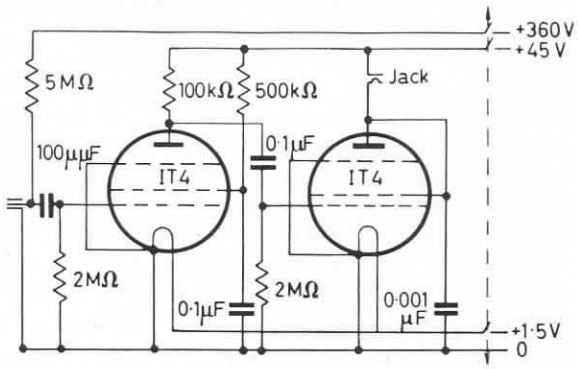

Figure 105. Circuit diagram of Geiger–Müller detector unit (after GODFREY, 1954).

the process of detection it is an advantage to have a low density of marked leaves; a large concentration makes it difficult to distinguish marked individuals. Leaves could be readily detected up to 12 months after marking, but after this time the paint began to disappear from some leaves.

THE DISAPPEARANCE OF BEECH LEAVES

A pilot experiment was commenced in October, 1956, when 1,000 leaves were marked in a young beech (*Fagus sylvatica* L.) wood (Knott Wood) at Rothamsted. The soil of the sample site is a clay with flints (Winchester series) overlying chalk some 60–90 cm (2–3 ft) below the surface. The humus form is more or less of mull type. There is no H layer, but a feebly developed F layer may be found in places, and from observation it would seem that leaf litter takes about one year to disappear from the forest floor. This wood is of the *Fagetum rubosum* type (TANSLEY, 1939) with *Rubus fruticosus* L. agg. and abundant *Endymion nonscriptus* (L.). The trees were planted in the winter of 1940–41, but growth has been somewhat retarded and, as a result, canopy in the sample plot has formed only recently.

The leaves were marked on the trees on 30th October, a few days before leaf fall. During the next 12 months they were sampled at intervals, photographed and oven-dry weights determined. Rainfall for the first six months

* Supplied by 20th Century Electronics Ltd., Centronics Works, New Addington, Croydon, Surrey.

(Nov. to Apr.: 10·9 in.; 277 mm) was 80 per cent of the long-term average. November and April were exceptionally dry, and December and February, appreciably wetter than average. The period May to October (14·6 in.; 371 mm) more closely resembled the normal rainfall pattern.

Sampling indicated that litter disappearance during the first six months was very slow indeed, but it is probable that there was a marked acceleration during the second six months. This bears out general impressions of the rate of disappearance of beech, which has a reputation for a somewhat slow rate, certainly very much slower than broadleaf species such as ash (*Fraxinus excelsior* L.), alder (*Alnus glutinosa* L.), and hornbeam (*Carpinus betulus* L.). However, weather conditions may play an important part, and it would appear that these were somewhat exceptional during the first half of the experiment. For this reason it would be unwise to generalize at this stage of the investigation.

The position of the leaf in the litter profile seemed to have an important bearing on leaf disintegration. As a rule, those at the base of this layer disappeared more rapidly, and this fact might be connected with the moisture status of the material. It was found that distribution was much affected by wind, and there was a tendency for marked leaves to occur grouped together in small pockets, or around an obstacle which had prevented further scattering.

Leaves were also examined to identify the fauna associated with them. One interesting feature was the presence of 'tyroglyphid' mites (Acaridiae) on samples obtained in April, 1956. The period preceding the sampling was very warm and dry—the rainfall for March (1·26 in.; 32 mm) was two-thirds of normal—and might account for their presence. This is believed to be the first definite observation of the occurrence of these mites in the L layer of the forest floor.

DISCUSSION

The principal advantages of this technique are: the leaves are maintained in conditions closely resembling those occurring in the natural milieu; the exact position of the leaf in the litter profile can be determined; and each marked leaf can be readily identified. The method may not be suitable for very small leaves such as conifer needles, but a smaller spot, 4·8 or 3·2 mm diameter ($\frac{3}{16}$ or $\frac{1}{8}$ in.) for example, with a greater concentration of isotope to provide an equivalent level of specific activity, might be adequate for small-leaved, deciduous species. A further limitation arises when the recovery period continues for more than 12 months. Here it would be necessary to use an isotope with a longer half life, and a more durable paint as adhesive. Cobalt 60 (half life: 5·25 years) might be a suitable substitute for ^{182}Ta, and the use of latex (polyvinyl acetate) or polystyrene paints might provide the answer to the latter problem.

Certainly the ideal labelling technique is one in which an isotope is incorporated in the leaf tissue, and there is no doubt that such a method would provide much valuable information. The isotope might be applied in a number of ways, one possibility being by injection. This is a rather complex problem, however, with a number of difficulties, not least being the ready leaching of ions from leaves, not only on the ground, but also while they are still on the tree. The use of carbon 14 might offer a solution but its use raises considerable practical difficulties.

An important aspect so far unconsidered is the effect of the isotope on the living inhabitants of the leaf litter. The principal danger may be its effect on reproductive capacity. BANKS (1955) found that newly-laid eggs of *Propylea* 14-*punctata* (L.) did not hatch when ^{182}Ta labels (7·5 μc) were placed in their vicinity. First instar larvae of *Coccinella* 7-*punctata* L. were also adversely affected by similar doses, but 1·3-μc labels had no noticeable effect on growth or behaviour. With some larger insects such as the Common Furniture beetle (*Anobium punctatum* (Deg.)), Death-watch beetle (*Xestobium rufovillosum* (Deg.)) and Powder-post beetle (*Lyctus brunneus* Steph.), arrested development of larvae and sterilization of adults can occur with comparatively low doses of the order of 8,000 r (^{60}Co source; BLETCHLY, 1957; 1958). Eggs, however, of the first two insects proved very resistant to gamma radiation. In preliminary experiments to find the amount of gamma radiation required to cause sexual sterility in mites of stored products (Acaridiae), CUNNINGTON (1957) found that *Acarus siro* L. (*Tyroglyphus farinae* Deg.), *Glycyphagus destructor* (Schr.) and *Tyrophagus castellanii* (Hirst) did not appear to be affected by doses of 20,000 r (^{60}Co source) and continued to reproduce normally; the last-named species was apparently little affected by a dose of 50,000 r. AUERBACH, CROSSLEY, and ENGELMAN (1957), in a recent study of the effect of gamma radiation (^{60}Co source) on population growth of *Proisotoma minuta* Tullb. (Collembola), found significant differences ($P = 0{\cdot}001$) between untreated populations and those exposed to 5,000 and 7,000 r. The population-growth curves with all treatments, and the control, had an initial threshold period followed by a typical phase of exponential growth. The chief effect of radiation (up to 5,000 r) appeared to be a lengthening of the threshold period. Nematodes are frequently associated with decaying leaves and it is interesting to note that doses of 48,000 to 96,000 r (^{60}Co source) adversely affected the reproductive capacity of *Ditylenchus destructor* Thorne and *Rhabditis* spp. (WOOD and GOODEY, 1957).

Non-spore-forming bacteria are less resistant to radiation than spore formers. DUNN, CAMPBELL, FRAM, and HUTCHINS (1948), for example, found that a dose of 35,000 r (X-ray source) killed 64 to 99 per cent of the former while doses of more than 500,000 r were required for the destruction of all bacterial spores. With fungi, Dunn, *et al.* found that lethal doses for *Aspergillus niger* van Tiegh (suspended in broth) and *Mucor* sp. were respectively about 250,000 and about 500,000 r. The experiments of MCLAREN, RESHETKO, and HUBER (1957) indicate that doses of about two million rep* (electron-beam source; beta and X-rays) will kill all bacteria and actinomycetes in soil, and the fungal fraction will succumb to a dose of about half this level. STAPLETON and MARTIN (in HOLLAENDER, 1955) rank ionizing radiations for killing *Aspergillus terreus* Thom (dry spores) in order of decreasing effectiveness as follows: neutrons and alpha rays > X-rays and gamma rays. It would seem likely therefore that rather higher levels of gamma radiation would be required to obtain a complete kill.

With the marked leaves, the maximum dose of gamma radiation (the

* Röntgen-equivalent-physical is defined as the dose of radiation which, when delivered to a given mass of tissue, produces the same real energy conversion as one röntgen delivered to the same mass of air (*ca.* 84 erg/g). This corresponds to an energy absorption of about 93 erg/g of tissue (assuming unit density).

beta radiation from ^{182}Ta is not considered here) would be very much less than the levels quoted above. Take for example the situation where an animal rests on the spot of paint and completely covers it. With a paint spot of 6·4 mm diameter and an initial activity of 4·9 μc, the organism would be exposed to about 3 r/h (assuming a distance of 1 mm from the source), and would have to occupy this position continuously for about 14 days for the dose to reach 1,000 r. Again, a colony of 42 mites (*Platynothrus peltifer* (C. L. Koch) Oribatei) would cover the spot (assuming that the silhouette of this species approximates to an ellipse with axes of 620 μ and 330 μ), and would be exposed to about the same level of gamma radiation. In practice it is unlikely that the 3-r radiation level is reached, due to the fact that the tantalum powder tends to settle out of the paint, so that the actual activity per spot is probably less than the calculated value. This fact coupled with the small amount of beech litter eaten by animals during the first three months after leaf fall makes it unlikely that the radiation will be more than half the quoted value. It is unlikely therefore that the radiation hazard will be serious for the meio- or macro-fauna. Fungi and micro-organisms are much more likely to be present on fresh leaves, and for these the initial value will apply; but as present evidence suggests that they can tolerate high doses, this is unlikely to affect the experimental method materially.

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